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Effects of Immunosuppression on Avian Coccidiosis: Cyclosporin A but Not Hormonal Bursectomy Abrogates Host Protective Immunity

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The effects of cyclosporin A (CsA) treatment and hormonal bursectomy on *Eimeria tenella* infection of chickens were investigated to evaluate the role of humoral antibody and cell-mediated immunity (CMI) in the host protective immunity to an intestinal protozoan disease, coccidiosis. Hormonal bursectomy had no significant effect on the host response to *E. tenella*. CsA treatment had a differential effect on the course of disease depending on how CsA was given relative to infection. Daily administration of CsA for 7 days beginning 1 day before primary infection with *E. tenella* enhanced disease resistance, whereas a single dose of CsA given before primary infection enhanced disease susceptibility compared with that of untreated controls. Chickens treated with CsA during the primary infection were resistant to reinfection at 5 weeks post-primary infection. Treatment of chickens immune to *E. tenella* with CsA at the time of secondary infection abrogated their resistance to reinfection despite the presence of high levels of coccidia-specific secretory immunoglobulin A and serum immunoglobulin G. Splenic lymphocytes obtained after CsA treatment demonstrated a substantially depressed concanavalin A response, but not a depressed lipopolysaccharide response. Because CsA was not directly toxic to parasites in vivo when administered during the secondary infection, these results suggest that CsA interacts with the immune system to allow priming during the primary infection, while interfering with the effector function of CMI during the secondary infection. Taken together, present findings indicate that CMI plays a major role in host protective immunity to *E. tenella*.

Coccidiosis is an intestinal protozoan infection caused by any of eight species of the genus *Eimeria*. Much work has characterized the immune response elicited by *Eimeria* spp. and identified the means by which such responses are translated into protective immunity. Although earlier investigations implicated the role of humoral factors in antiparasitic immunity (reviewed in reference 22), recent investigations in agammaglobulinemic chickens (7; H. S. Lillehoj, Poult. Sci. 65:80, 1986) suggest that antibodies do not play a major role in the development of protective immunity. The importance of cell-mediated immunity (CMI) in the development of protective immunity to coccidiosis has been suggested in mice (20), but there is, as yet, no convincing in vivo model system to study the role of T-cell-mediated immunity in avian coccidiosis.

Cyclosporin A (CsA), a neutral, hydrophobic, cyclic, fungal metabolite, is a well-known immunosuppressive drug that interferes at an early stage with the activation of resting lymphocytes by inhibiting the expression of the gene for interleukin-2 (14, 16), the expression of the interleukin-2 receptor antigen (18), and the production of other lymphokines (24). The early studies of Borel and co-workers (2) clearly established that CsA is an immunosuppressive agent that selectively acts on a defined subpopulation of immunocompetent T cells. Much of the information concerning the action of CsA is based on in vitro experimental systems; thus, the immunoregulatory role of CsA in vivo has not been thoroughly investigated. Recent suggestions that CsA has a different mode of action in vivo than in vitro (13) compel further investigation on the mechanism of immunomodulation mediated by CsA in vivo.

The present study was undertaken to investigate the immunoregulatory role of CsA in vivo and the effect of CsA treatment and hormonal bursectomy on primary and secondary infections with *Eimeria tenella*. Two different inbred strains of chickens, genetically showing different disease susceptibility to *E. tenella* (19), were used to rule out the possibility that the CsA-mediated immunomodulation of coccidial infection was dependent on the host genetic background. Three different regimens of CsA treatment were investigated: (i) administration during only the preinfection phase, (ii) administration during only the induction and clinical phase of immunity, and (iii) administration only on the established acquired resistance. This report describes the differential immunological effects that CsA has on the primary and secondary infections with *E. tenella* depending on the treatment regimen.

MATERIALS AND METHODS

Chickens. Embryonated SC (B2/B2) and FP (B15/B21) chicken eggs were obtained from Hy-line International Production Center (Dallas Center, Iowa). All chickens were housed in clean wire-floored cages and provided feed and water ad libitum. Chickens that were 4- to 6-weeks-old were used in all experiments.

Development of agammaglobulinemic and dysgammaglobulinemic chickens. Hormonal bursectomy was done by combining in ovo treatment with testosterone propionate (Sigma Chemical Co., St. Louis, Mo.) and cyclophosphamide (Sigma) injection at hatching as described previously (8). On day 12 of embryonation, each egg was injected in the allantoic sac with 0.1 ml of sterile corn oil containing 4.0 mg of testosterone propionate. At 1 and 2 days of age, each chick was intraperitoneally injected with 4.0 mg of cyclophosphamide dissolved in ethanol-saline. Hormonally bursectomized chickens were tested for the presence of serum immunoglobulin G (IgG), IgM, and IgA at 4 weeks of age by Ouchterlony immunodiffusion and enzyme-linked immunosorbent assay (ELISA) as described below. Agammaglobulinemic chickens with no detectable immunoglobu-

lin were unable to produce anticoccidial antibodies upon infection with *E. tenella*. Dysgammaglobulinemic chickens were deficient in at least one subclass of immunoglobulin and produced anticoccidial antibody of only certain subclasses upon infection with *E. tenella*.

Parasites and inoculation of birds. The strain of *E. tenella* used (LS24) was developed from a single oocyst isolation and maintained at the Animal Parasitology Institute (Beltsville, Md.). Ten 4-week-old chickens were inoculated orally with 10^4 sporulated oocysts of *E. tenella*. This inoculating dose gave an optimum oocyst production in both SC and FP chickens in the previous study (H. S. Lillehoj, Poult. Sci. 65:79, 1986). Four consecutive daily oocyst counts for individual birds began 5 days post-primary inoculation (p.p.i.) using a McMaster counting chamber. Because a previous investigation showed that chickens develop protective immunity starting at 3 weeks p.p.i. (17), secondary inoculation was done by inoculating immune birds with 10^4 oocysts at 5 weeks p.p.i. Four consecutive daily oocyst counts were made in the individual birds beginning 6 days post-secondary inoculation (p.s.i.).

Lesion score and PCV determination. Chickens show severe cecal lesions and a significant decrease in packed-cell volume (PCV) upon infection with *E. tenella*. Cecal lesion score was assessed by the previously described method (10) at 5 days p.p.i. PCV was determined at 5 days p.p.i. in birds infected with *E. tenella* as described previously (19).

ELISA. The previously described procedure for ELISA (19) and the optimum concentrations of antigen, antisera, and substrate were used to assess antibody response to coccidia.

To determine the presence of immunoglobulins in chickens that were hormonally bursectomized, I tested sera obtained from chickens in the ELISA. Antigen-coated plates were prepared by coating plates with optimum concentrations of rabbit anti-chicken IgG or goat anti-chicken IgM or IgA specific for each of the heavy chains of chicken immunoglobulins (Miles Laboratories, Inc., Elkhart, Ind.).

Antigen-coated plates were treated with 10% bovine serum albumin for 2 h at room temperature to block nonspecific binding sites. Serum to be tested was serially diluted twofold. A 50-μl sample of chicken serum to be tested was added to antigen-coated wells. After 2 h of incubation at room temperature, the plates were washed four times with the washing buffer (phosphate-buffered saline containing 0.05% Tween 20). Rabbit anti-chicken IgG (50 μl) (Miles) was added, and plates were incubated for 1 h at 37°C. The plates were washed four times and then incubated with 50 μl of biotin antirabbit antiserum (Sigma). After 30 min of incubation at 37°C, the plates were washed and incubated with 50 μl of streptavidin-peroxidase (Zymed, San Francisco, Calif.). Enzyme reactions were initiated by the addition of *ortho*-phenylenediamine dihydrochloride dissolved in 0.05 M citrate phosphate buffer containing fresh H_2O_2. Optical density was read at 450 nm with a multichannel spectrophotometer (Titertek Multiskan; Flow Laboratories, Inc., McLean, Va.).

In vivo treatment with CsA. To investigate the effects of immunosuppression of CMI on the host response to coccidia, I treated chickens with CsA. CsA dissolved in olive oil at 100 mg/ml was used to treat chickens. Two different regimens were used to investigate the effect of CsA treatment on the primary infection of *E. tenella*. In one group, chickens were treated 1 day before primary infection with 0.5 ml of CsA per os, followed by infection with either 10^5 or 10^4 sporulated oocysts of *E. tenella*. Another group of chickens was treated with 0.5 ml of CsA for 7 consecutive days beginning 1 day before primary infection. Controls were inoculated with olive oil alone. To investigate the effect of CsA on secondary infection, I treated chickens daily with 0.5 ml of CsA for 2 weeks beginning 1 day before secondary inoculation. These chickens were challenged with 10^4 sporulated oocysts of *E. tenella* at 5 weeks p.p.i. Each group consisted of six 4-week-old SC and FP chickens.

In vitro mitogen-induced lymphoproliferation assay. Single-cell suspensions were prepared from spleens obtained from control or CsA-treated chickens by teasing spleen cells in Hanks balanced salt solution. Mitogen-induced stimulation was performed by incubating various concentrations of splenic lymphocytes with different concentrations of concanavalin A (ConA) (Pharmacia, Inc., Piscataway, N.J.) or lipopolysaccharide (LPS) (Sigma) in 96-well microtiter plates in 0.2 ml of complete medium for 3 days. Complete medium consisted of RPMI 1640 medium supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (5×10^{-5} M), HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (10 mM), and 5% fetal calf serum. Cell cultures were incubated at 41°C in a humidified atmosphere of 6% CO_2 in air. Twenty hours before harvesting, 1 μCi of tritiated thymidine (New England Nuclear Corp., Boston, Mass.) was added to each well. The cultures were harvested with a PHD cell harvester (Cambridge Technology, Inc., Cambridge, Mass.), and the amounts of radioactivity associated with cellular DNA were measured with a β-scintillation counter (Packard Instruments Co., Inc., Rockville, Md.).

Statistics. The Mann-Whitney U test (25) was used to test for differences among groups.

RESULTS

Effect of hormonal bursectomy on primary infection with *E. tenella*. To determine the effect of immunosuppression of humoral response on avian coccidiosis, I rendered chickens B-cell deficient by hormonal bursectomy. The efficiency of hormonal bursectomy depended on the dose and treatment regimens. In general, treatment of SC and FP chickens with testosterone in ovo and with cyclophosphamide at hatching resulted in agammaglobulinemia in only 10% of hormonally bursectomized chickens (data not shown). These agammaglobulinemic chickens did not produce anticoccidial antibodies at 14 days p.p.i., suggesting that they were truly agammaglobulinemic (data not shown). A total of 50% of hormonally bursectomized chickens lacked one or two immunoglobulin isotypes. The remaining 40% had detectable IgG, IgM, and IgA and produced high levels of anticoccidial antibodies upon infection with *E. tenella*. Normal and immunoglobulin-deficient chickens (4 weeks old) were infected with 10^5 oocysts of *E. tenella*, and disease susceptibility was assessed by determining the PCV at 5 days p.p.i. (Table 1). PCV was measured since a previous investigation showed that it provides a sensitive measurement of the severity of infection (19). In general, SC chickens that were hormonally bursectomized (group 2) had lower PCVs than untreated chickens (group 1) despite the presence of all three types of immunoglobulins (Table 1) ($P < 0.05$, Mann-Whitney U test; level of significance for two-tailed test, $\alpha = 0.05$). Agammaglobulinemic (group 3) and dysgammaglobulinemic (groups 4, 5, and 6) chickens did not differ significantly with respect to disease susceptibility. These results suggest that B-cell immunodeficiency does not influence the outcome of primary infection with *E. tenella*.

Oocyst production in agammaglobulinemic chickens after primary and secondary infections. Ten 4-week-old normal and agammaglobulinemic chickens were inoculated with 10^4 oocysts, and oocysts were counted to investigate effects of serum and secretory immunoglobulins on disease susceptibility and the acquisition of resistance to reinfection (Fig. 1). A previous study showed that an inoculating dose of 10^4 oocysts gives optimal oocyst production in SC and FP chickens (Lillehoj, Poult. Sci. 65:80, 1986). There were no significant differences in oocyst production between normal and agammaglobulinemic chickens ($P > 0.05$) after primary infection. Furthermore, both normal and agammaglobulinemic chickens were resistant to reinfection upon secondary inoculation on the basis of oocyst production. No significant differences in the duration of the prepatent or patent period were noted between normal and hormonally bursectomized groups, although hormonally bursectomized birds had a higher mortality (data not shown).

Effect of CsA treatment on primary inoculation with *E. tenella*. Because bursectomy studies suggested that humoral immunity does not influence disease, I decided to investigate the effects of CMI on disease using the immunosuppressive drug CsA. To investigate the effects of CsA treatment on the course of coccidiosis upon primary infection, I treated six 4-week-old FP chickens with CsA. Two different treatment regimens were used: one treatment before the inoculation or seven daily treatments starting 1 day before the inoculation. Chickens pretreated once with CsA 1 day before primary infection showed generally produced more oocysts (Fig. 2). In contrast, daily treatment with CsA for 1 week beginning 1 day before primary infection significantly reduced the numbers of oocysts produced ($P < 0.05$). Taken together, these results suggest that prolonged CsA treatment of birds significantly enhances disease resistance, whereas a single pretreatment of birds before primary infection enhances disease susceptibility.

Effect of CsA treatment on secondary infection. To investigate the effects of CsA on the host protective immunity to coccidiosis, I used two different treatment regimens. Groups in Fig. 3 designated as a received daily CsA treatment for 1 week beginning 1 day before the primary infection. Secondary inoculation was given at 5 weeks p.p.i. Groups designated as b received daily CsA treatment for 1 week beginning 1 day before the primary infection and for 2 weeks beginning 1 day before secondary infection. Each group consisted of six 4-week-old chickens. SC and FP chickens not treated with CsA at the time of secondary infection did

TABLE 1. Effect of hormonal bursectomy on primary infection with *E. tenella*

Group	Strain	Treatment[a]	IgG[b]	IgA[b]	IgM[b]	PCV ± SD[c]
1	SC	N	+	+	+	34 ± 1*
	FP	N	+	+	+	24 ± 1†
2	SC	B	+	+	+	23 ± 2‡
	FP	B	+	+	+	28 ± 4†
3	SC	B	−	−	−	28 ± 5‡
	FP	B	−	−	−	27 ± 7†
4	SC	B	+	−	−	23 ± 6‡
	FP	B	+	−	−	23 ± 11†
5	SC	B	−	+	−	25 ± 1‡
	FP	B	−	+	−	19 ± 8†
6	SC	B	+	+	−	24 ± 1‡
	FP	B	+	+	−	23 ± 1†

[a] Four-week-old normal (N) or hormonally bursectomized (B) chickens (20 per group) were inoculated with 10^5 oocysts of *E. tenella* (LS 24).
[b] The presence of IgG, IgM, or IgA serum immunoglobulin was determined by Ouchterlony immunodiffusion at 4 weeks of age.
[c] PCV was determined at 5 days postinfection. The average results from two independent experiments is shown. Groups with same symbol from the same strain are not statistically different, while those with different symbol are statistically different.

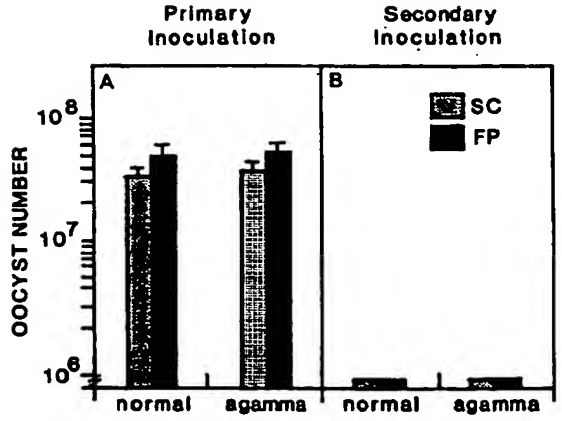

FIG. 1. Oocyst production in normal and agammaglobulinemic chickens upon primary and secondary infection. (A) Ten 4-week-old SC and FP chickens were inoculated with 10^4 sporulated oocysts. Oocyst production was measured during the patent period from days 5 to 10 p.p.i. (B) Ten immune SC and FP chickens were infected with 10^4 sporulated oocysts 5 weeks p.p.i. Oocyst production was measured for 4 days starting 6 days p.s.i.

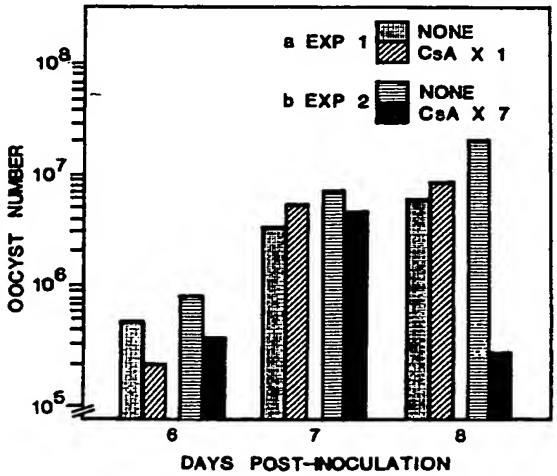

FIG. 2. Effect of CsA treatment on primary infection with *E. tenella*. (a) In experiment 1, six 4-week-old FP chickens were treated with CsA (100 mg/kg of body weight) at 1 day before primary infection. (b) In experiment 2, six 4-week-old FP chickens were treated with CsA for 7 consecutive days starting 1 day before primary infection. Oocyst counts were measured on individual birds beginning 5 days p.p.i. No oocysts were seen during the first 5 days after primary infection.

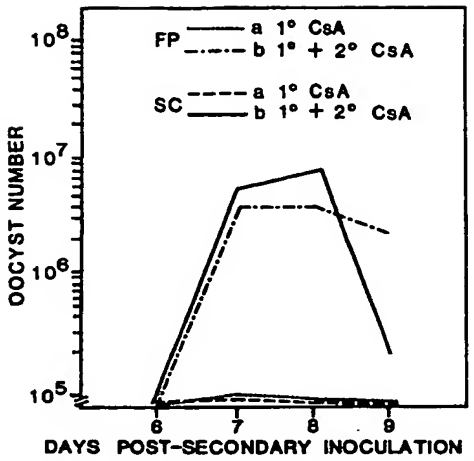

FIG. 3. Effect of CsA treatment on secondary infection with *E. tenella*. Six age-matched SC and FP chickens were infected with 10^4 oocysts of *E. tenella*. Groups designated as a were treated with CsA for 1 week beginning 1 day before primary infection. These birds were reinfected with 10^4 oocysts at 5 weeks p.p.i. No CsA was given to these groups at the time of secondary infection. Groups designated b received CsA treatment at the time of primary and secondary infection. Chickens were treated with CsA for 2 weeks beginning 1 day before secondary infection.

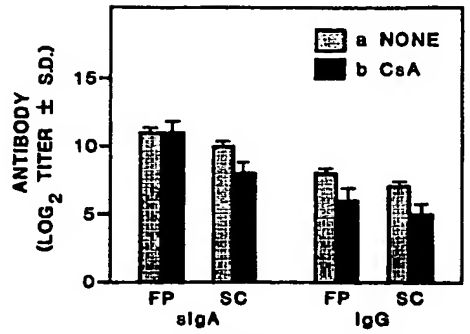

FIG. 4. Anticoccidial antibodies in normal and CsA-treated chickens. FP and SC chickens were infected twice with 10^4 oocysts of *E. tenella* at 5 weeks apart. Chickens in group a received no CsA treatment. Chickens in group b were treated with CsA at the time of primary and secondary infection. Each group consisted of six 4-week-old chickens. Chickens were treated with CsA as described in the legend to Fig. 3. Sera and bile secretions from these chickens were collected at 5 days p.s.i. Secretory IgA (sIgA) and serum IgG were determined by ELISA. S.D., Standard deviation.

not produce oocysts upon secondary infection. In contrast, chickens treated with CsA during the secondary inoculation produced significant numbers of oocysts at 7, 8, and 9 days p.s.i. No mortality occurred in CsA-treated chickens, indicating that the CsA treatment given in this study was not lethal. Groups of chickens that were not treated with CsA during both the primary and secondary infections were resistant to challenge infection (data not shown). These results suggest that CsA treatment of chickens at the time of secondary infection abrogates the host protective immunity.

Anticoccidial antibodies in CsA-treated birds challenged with *E. tenella*. To assess the effect of CsA treatment on the production of antibodies to *E. tenella*, I tested immune sera and bile secretions obtained from CsA-treated immune chickens using ELISA. Both CsA-treated and non-CsA-treated immune chickens showed high levels of anticoccidial IgG and biliary secretory IgA antibodies (Fig. 4). This result suggests that CsA-mediated abrogation of protective immunity is not due to the immunosuppression of anticoccidial humoral response.

Effect of CsA treatment on ConA- and LPS-induced lymphoproliferation. Since CsA treatment of chickens caused marked modulation of host response to coccidiosis, the effect of peroral treatment of CsA on splenic T- and B-cell function was tested. Chickens treated with CsA once at 1 day before assay or for 1 week beginning at 1 day before assay by daily inoculation of CsA showed a diminished ConA response but not a diminished LPS response (Table 2). Although high concentrations of CsA (up to 1 μg/ml) inhibited the LPS-induced lymphoproliferation response of normal splenic lymphocytes in vitro (data not shown), the lack of CsA inhibition of LPS-induced lymphoproliferative responses of splenic lymphocytes from CsA-treated chickens suggested that the dose of CsA used in the present study was not inhibitory for the LPS-induced B-cell response. These results suggest that the dose of CsA used in the present study had a preferential inhibitory effect on T-cell response but not on B-cell response.

DISCUSSION

The results of the present investigation strongly suggest that CMI, rather than humoral immunity, is responsible for the development of a protective immune response against *E. tenella* infection. This conclusion is based on the following observations. (i) Hormonally bursectomized agammaglobulinemic chickens show no difference in oocyst production and PCV upon primary infection or in resistance to secondary infection with *E. tenella*, compared with untreated control chickens. (ii) Oral treatment with CsA caused preferential depression of the splenic mitogenic response to ConA, a T-cell mitogen, but not to LPS, a B-cell mitogen. (iii) Daily administration of CsA to chickens immune to *E. tenella* at the time of secondary inoculation abrogated protective immunity despite the presence of high levels of secretory and circulating antibodies specific for *E. tenella*.

Infection of chickens with *Eimeria* species is accompanied by the production of antibodies (19) and the development of parasite-antigen-specific CMI (17). The role of antibodies in antiparasitic immunity to avian coccidiosis remains contro-

TABLE 2. Effect of CsA treatment on ConA- and LPS-induced lymphoproliferation

Expt	Treatment[a]	[^{3}H]thymidine uptake (Δcpm ± SD)[b] with:	
		ConA	LPS
1	None	31,921 ± 905	19,558 ± 560
	CSA × 1	490 ± 252	26,140 ± 469
2	None	10,734 ± 205	9,781 ± 350
	CSA × 7	3,709 ± 25	9,987 ± 212

[a] Ten 4-week-old SC chickens were treated with CsA once at 1 day before the assay (CSA × 1) or daily for 1 week beginning 1 day before the assay (CSA × 7). No mortality associated with CsA treatment was observed in CsA-treated groups.

[b] Δcpm, Counts per minute with ConA (10 μg/ml) or LPS (100 μg/ml) − counts per minute medium.

versial. Although an earlier study on bursectomized chickens (7) suggested that antibodies were not essential, it failed to rule out the possible role of maternal antibodies in the primary infection with coccidia since chickens were infected at 2 weeks of age. In the present study, 4-week-old chickens were used to rule out any involvement of maternal antibodies. Furthermore, the present investigation indicates that not all hormonally bursectomized chickens lack circulating immunoglobulins. An additional finding that was not discussed in the earlier study is that some of the hormonally treated chickens that are not agammaglobulinemic are more susceptible to coccidiosis compared with nonbursectomized control chickens upon primary infection. Taken together, the results of the present study demonstrate that antibodies play a negligible role in determining disease susceptibility and the development of protective immunity to coccidiosis.

It has been well documented that chickens will develop a good protective immunity against coccidia after infection with the live parasites. Although work on the nature of immunity to coccidiosis and the immune mechanism(s) involved in the mediation of resistance to reinfection have been intensively studied over the past 20 years, the mechanism(s) of the development of protective immunity is not well understood. The importance of CMI has been suggested by the adoptive transfer of protective immunity with immune lymphocytes (23) and the development of potent T-cell responses to sporozoite and merozoite antigens in immune chickens (17). In this study, CsA, a drug with well-characterized cell-mediated immunosuppressive activity (2, 16), was used to define further the involvement of T-cell-dependent mechanisms in avian resistance to coccidiosis. Although the effects of oral CsA treatment on the intestinal immune system of chickens were not investigated, the splenic lymphocyte response to ConA was severely depressed compared with that in untreated control chickens. The importance of a T-cell-dependent mechanism(s) in antiparasitic immunity to coccidia was further demonstrated by the significant numbers of oocysts produced upon secondary infection in chickens treated with CsA. Interestingly, these chickens showed high levels of coccidia-specific secretory IgA and serum IgG, suggesting a minimal role of antibodies in conferring disease resistance. Infecting coccidia do not appear to be directly sensitive to CsA at the physiologically achievable concentration used in the present study since CsA-treated but not untreated chickens shed significant numbers of oocysts upon secondary infection. This is in contrast to malaria, which was shown to be sensitive to CsA (21).

The differential effect of CsA on primary infection with *E. tenella* depending on the length of treatment was not expected but proved to be consistent. Although the mechanism for this phenomenon cannot be explained at present, the results of the present study suggest that CsA exerts different immunopharmacological effects depending on when it is given with respect to antigen priming. Chickens treated with CsA for 1 week beginning 1 day before primary infection were resistant to the secondary infection, suggesting that antigen-specific T-cell priming and clonal expansion occurred despite the CsA treatment. These results seem confusing in view of the fact that CsA is an immunosuppressive drug that is effective in preventing allograft rejection (5, 6), autoimmune reactivity (29), and graft-versus-host disease (28). CsA-mediated enhancement of resistance upon primary infection in CsA-treated chickens may reflect a very complex pattern of events occurring at the induction phase of the immune response toward *Eimeria* species which is somehow altered by CsA. Whether the immune enhancement mediated by CsA is due to the effect of CsA on suppressor cells, similarly suggested in OS chickens (29), or due to inhibition of the anti-inflammatory response mediated by CsA (11) remains to be elucidated. The enhanced resistance conferred by CsA treatment upon primary infection may have an immune basis similar to that induced by CsA pretreatment of other parasitic infections (1, 3, 4). In *Leishmania major* infection in mice (1), CsA treatment had a striking prophylactic effect, and these mice were capable of developing protective immunity. Similar phenomena were described during *Schistosoma mansoni* infections in mice (2, 4). CsA treatment was not only effective in eliminating the infecting worms during the primary infection but also induced a long-term protective effect.

Although CsA has been widely used in patients to prevent allograft rejection, much about the in vivo action of CsA remains unknown. Recent investigations suggest that CsA has somewhat unpredictable effects on the immune systems of animals and humans (13, 26). Some of the effects of CsA in vivo, such as immune enhancement (15, 27) and exacerbation of experimental autoimmune thyroiditis in chickens (29) and *Trypanosoma cruzi* infection in mice (12), need further investigation. The continued in vivo study of CsA and its interactions with the immune system in different experimental systems will shed considerable light on the immunoregulatory pathways which determine the outcome of an immune response toward both infectious and noninfectious antigens.

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